RESEARCH ARTICLE Am. J. PharmTech Res. 2012; 2(5) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Polymeric Film Devices as Transdermal Therapeutic Systems for Diabetes.

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ABSTRACT

The purpose of the present investigation was to enhance the dissolution properties and bioavailability of a poor water soluble drug (Glibenclamide) by matrix-type transdermal films. Eudragit S 100 (Ed S100) and Hydroxypropylmethyl cellulose (HPMC) were used individually or in mixtures to formulate the transdermal devices by solvent casting method. The physicomechanical evaluation of the polymer matrices was performed. The prepared films showed good physicomechanical characteristics as Ed S100 percent increased, where HPMC lowered these characters. In-vitro release studies of ideal films were evaluated and the results revealed that the amount of Glibenclamide released was affected to great extent by the polymer type and also the solvent used. HPMC films showed high release rates of the drug where Ed S100 retards and control the drug release rates. HPMC mixtures with Ed S100 in 1:1 ratio released about 100% of the drug after 6 hours. In vivo evaluation of the prepared films was investigated by testing the hypoglycaemic effects of the drug after transdermal application to diabetic rats. The observed results simulated that obtained after oral administration of Glibenclamide in a dose of 5 mg/kg. The obtained results revealed a good promise of using Glibenclamide via transdermal route which could be of excellent benefit when oral route is unavailable.

Keywords: Glibenclamide, Film devices, Tensile strength, Transdermal, Blood Glucose.

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Received 25 June 2012, Accepted 11 July 2012

Please cite this article in press as: Mokhtar M *et al.*, Polymeric Film Devices as Transdermal TherapeuticSystems for Diabetes.. American Journal of PharmTech Research 2012.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level. In total diabetic patients more than 90% are suffering from type II diabetes[1]. Type II diabetes is mainly due to insufficient insulin production in the body caused by partial or incomplete inactivation /destruction of β cells of pancreas and often associated with insulin resistance syndrome [2,3]. The treatment of type II diabetes includes life style changes, drugs that reduce intestinal glucose uptake and hepatic gluconeogenesis and drugs that increase insulin secretion from the pancreas (*e.g.* sulfonylureas). Glibenclamide, a sulfonylurea, has short term and long term pharmacological actions. During short term treatment it increases insulin secretion from functioning pancreatic islet β cells (pancreatic effect) where as during long term treatment its main action appears to be enhancement of insulin action on peripheral tissues and reduction of glucose output from the liver (extra-pancreatic effect). In short term treatment, Glibenclamide causes de-granulation of the β cells in pancreas[4]. The evoked release of insulin from the pancreas is very rapid and sulfonylureas appear to stimulate calcium influx into islet cells[5,6] . Glibenclamide is a lipophilic molecule and acts on sulfonylurea receptors within the hydrophobic phase of the cell membrane[7]. It is practically insoluble in water which leads to poor dissolution rate and subsequent decrease of its gastrointestinal (GI) absorption. Several investigations had revealed that the absorption of Glibenclamide was limited by its dissolution rate[8,9]. The administration of Glibenclamide via transdermal therapeutic system (TTS), may provide several advantages over oral route such as controlled release of the active pharmaceutical ingredient (API), minimized plasma fluctuation of the drug, elimination of first pass metabolism, reduced the intensity of action and thus reduced the side effect (hypoglycemia) associated with its oral therapy and improved patient compliance [7,10] . Patient compliance could also increase because antidiabetic drugs have to be taken throughout the patient life[11]. The physicochemical properties of Glibenclamide are very suitable for a TTS, *i.e.*, molecular weight 494 Da, variable biological half life 0.1-10 h, an effective plasma concentration 30-50 μg/L and a reasonable partition coefficient (octanol/water) 4.23[12,13]. In the present study, the matrix type of TTS for Glibenclamide delivery was successfully formulated. A matrix type system was taken into consideration because of the low dose of the drug. The TTS was prepared using varied ratios of Eudragit S 100 and HPMC in order to enhance the dissolution rate and the systemic absorption of poor water soluble drugs. The lower the oral dissolution and the poor bioavailability of Glibenclamide suggested the drug as a candidate for exploring its application as transdermal drug delivery system. Different plasticizers were also tested for their effects on the physicochemical properties of the prepared films, the rate of drug dissolution and their penetration enhancing properties.

Finally, the hypoglycemic effects of the drug were evaluated after application of the film devices on hyperglycemic rats and the pharmacokinetic parameters were also calculated

MATERIALS AND METHODS

Materials:

Glibenclamide and HPMC 2910 E5 were gifts from Egyptian Pharmaceutical Industrial Company (E.P.I.Co, Egypt). Disodium Hydrogen Phosphate and Potassium Dihydrogen Phosphate were purchased from El-Nasr Pharm. Chem. Company (Cairo, Egypt). Eudragit S100 was purchased from Merck pharm. Company (Germany). Methanol, methylene chloride, anhydrous calcium chloride, potassium chloride and acetone were purchased from El-Gomheria pharm. Company (Cairo, Egypt). Polyethylene glycols 200 and 400 (PEG 200 and PEG 400) were purchased from ADWIC pharm. Company (Cairo, Egypt). Triacetin was purchased from Goodrich Chemical Co. (England) and Alloxan monohydrate was purchased from Sigma Chemical Co. (St. Louis, USA).

Development of transdermal films

Matrix type transdermal films containing Glibenclamide were prepared by solvent casting method. Either 5 % w/w Ed S100 or 2.5 % w/w HPMC films were prepared by dissolving each polymer in the measured volume of the organic solvent of choice such as methanol or acetone to dissolve Ed S100 and Methylene chloride to dissolve HPMC polymer. Magnetic stirring of the solutions in tightly closed containers for overnight was done to ensure complete dissolution of the polymer in the solvent (Heating Magnetic Stirrer, Voltage 230, W 800, Europe). Methanol /methylene chloride mixture (1:1 ratio) was used for combined polymeric films. A clear polymeric solution was obtained after plasticizers were added in different percentages which used according to the required physical characters of the prepared films. Glibenclamide (0.1% w/w) was then dissolved in the prepared polymeric solutions. Then the drug polymer solutions were poured into glass Petri dishes and covered with an inverted glass funnel. The funnel was used to aid in controlling the rate of evaporation of the solvent and reducing the blistering of the surface of the deposited film. The solvent was allowed to evaporate for 24 hours after which the dry films was isolated, wrapped in an aluminum foil and stored in dessicator at ambient room temperature. Formulation composition of film matrices is reported in table 1. The obtained formulations were evaluated according to a rating system for viscosity, drying time, stickiness of the outer surface, cosmetically attractiveness and integrity on the skin after 18 hr.

Table 1. Formulation composition of film matrices.

Formula Code	Polymer	Plasticizer (% of polymer wt.)	Solvent	Drug (Glibenclamide)	Film
F1	Ed S 100(500 mg)	PEG 400(40 %)	Acetone	(10 mg)(0.1 %)	Formed
F2	Ed S 100(500 mg)	PEG 400(40 %)	Methanol	(10 mg)(0.1 %)	Formed
F3	Ed S 100(500 mg)	Triacetin(60 %)	Acetone	(10 mg)(0.1 %)	Formed
F4	Ed S 100(500 mg)	Triacetin(60 %)	Methanol	(10 mg)(0.1 %)	Formed
F5	Ed S 100(500 mg)	PEG 200(60 %)	Acetone	(10 mg)(0.1 %)	Formed
F6	Ed S 100(500 mg)	PEG 200(60 %)	Methanol	(10 mg)(0.1 %)	Formed
F7	HPMC(250 mg)	PEG 400(40 %)	Methylene chloride	(10 mg)(0.1 %)	Formed
F8	HPMC(250 mg)	Triacetin(60 %)	Methylene chloride	(10 mg)(0.1 %)	Not Formed
F9	HPMC(250 mg)	PEG 200(60 %)	Methylene chloride	(10 mg)(0.1 %)	Formed
F10	HPMC: Ed S 100 (150 mg : 350 mg)	PEG 400(40 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F11	HPMC: Ed S 100 (150 mg : 350 mg)	Triacetin(60 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F12	HPMC: Ed S 100 (150 mg : 350 mg)	PEG 200(60 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F13	HPMC: Ed S 100 (350 mg : 150 mg)	PEG 400(40 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F14	HPMC: Ed S 100 (350 mg : 150 mg)	Triacetin(60 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F15	HPMC: Ed S 100 (350 mg : 150 mg)	PEG 200(60 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F16	HPMC: Ed S 100 (250 mg : 250 mg)	PEG 400(40 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F17	HPMC: Ed S 100 (250 mg : 250 mg)	Triacetin(60 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed
F18	HPMC: Ed S 100 (250 mg : 250 mg)	PEG 200(60 %)	Methylene chloride: Methanol (1:1)	(10 mg)(0.1 %)	Formed

Evaluation of the physicomechanical properties

Thickness:

The thickness of the film at three different points was determined using digital caliber (Electronic digital Caliber, AHK, Germany) and average thickness was observed.

Folding endurance test:

Folding endurance test was carried out by folding the film at the same point a number of times till it broke [14].

Tensile strength and percentage Elongation:

The films were cut into strips of 1cm width and 6 cm length. The films were fixed onto the tensile strength apparatus (Hounsfield, Slinfold, and Horsham, U.K.) in such a way that the length of film between the jaws was initially 5 cm[15,16]. The tensile strength (T.S.) gives indication about strength and elasticity of the film and calculated using the following equations:

$$\textit{T}\text{ensile strength (kg/cm}^2\text{)} = \frac{\text{Breaking load}}{\text{Cross sectional area of the film}}$$

$$\text{Percent Elongation} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \text{X100}$$

Percentage Moisture Loss:

Accurately weighed films of each formulation (2.5 × 3.2 cm²) were kept in a desiccators containing fused anhydrous calcium chloride at room temperature and weighed after 3 days. The percent moisture loss can be calculated using the following equation:

$$\text{Percent moisture loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \text{X100}$$

Percentage moisture uptake (absorption):

Accurately weighed films of each formulation (2.5 × 3.2 cm²) were kept in a desiccator which is maintained at 84% relative humidity (by adding 100 ml of saturated solution of potassium chloride in the desiccator) at room temperature and weighed after 3 days[17]. The test was carried out in triplicate.

$$\text{Percent moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{X100}$$

Water absorption capacity*:*

Dry films were weighed accurately and then each film was placed in a Petri dish containing 15 ml distilled water. The films were weighed periodically at 1st hr, 2nd hr, 3rd hr and 24th hr. Every time after noting the weight, the film was placed in fresh water, water absorption capacity of the film was determined in triplicate.

$$\text{Water absorption capacity} = \frac{\text{Ww} - \text{Wd}}{\text{Wd}} \text{X100}$$

Where

W_w = weight of wet film, W_d = weight of dry film.

Water vapor transmission (WVT) rate:

Glass vials of equal diameter were used as transmission cells. About 1 gm of anhydrous calcium chloride was taken in the cell and 1 × 1 cm² polymer film was fixed over the brim with the help of an adhesive tape. The cell were accurately weighed and kept in a closed desiccator containing saturated solution of potassium chloride to maintain a humidity of 84% RH .The cells were taken out and weighed after 2, 8, 12, 24, 48, and 72 hours [18].

$$\text{WVT} = \text{W.L/S}$$

Where,

WVT is water vapor transmission rate, ,W is grams of water transmitted, L is thickness of the film in cm, S is the exposed surface area of the film in cm^2.

EVALUATION OF THE PHYSICOCHEMICAL PROPERTIES

Drug content:

Films of specified area (1×1 cm^2) were cut and the pieces were taken in sufficient quantity of methanol. The volume was made up to 10ml. A blank was prepared in the same manner using a drug-free placebo patch of same dimensions. Then drug content was analyzed spectrophotometerically (Genesys UV spectrophotometer, USA) at 230 nm for Glibenclamide [19].

Weight variation:

The weight of each film (1x1 cm^2) was measured using digital balance (Electric balance**,** Sartorius, capacity 210 gm, Germany) and the average was calculated [20].

Interaction studies

Interactions between the polymers and drug were analyzed by Fourier transform infrared (FT-IR) spectral analysis and differential thermal analysis (DTA) as follows:

A: FT-IR experiment:

FT-IR (Perkin Elmer, Rodgau, Germany) spectra of pure powdered Glibenclamide, Eudragit S100, HPMC, physical mixture of Glibenclamide: HPMC: Eudragit S 100 in ratio of (0.25:1:1) and dried medicated films were taken in the range of 400-4000 cm^{-1}. In which each formula (5 mg) was mixed with about 100 mg potassium bromide and compressed into discs under pressure of 10,000 to 15,000 pounds per square inch [21].

B: DTA study:

DTA (D-60, Shimadzu, Japan) analysis was used to assess the thermal behavior of the drug and polymers. The instrument was calibrated using purified indium (99.99%). 5 mg of each of drug , polymers, physical mixture of them and mediated film samples were sealed in a flat bottomed platinum cell (Shimadzu DTA-50, Japan) separately. The cell was placed in the DTA instrument and scanned up to 600 ºC at a rate of 10 ºC/min. dry nitrogen was used as a carrier gas to eliminate the oxidative and pyrrolytic effects with a flow rate of 20 ml/min . The melting and transition point measurements were performed using the software provided with the device[3,4]**.**

In vitro drug release studies

A rectangular film of (2.5 × 3.2 cm^2) size was cut using a glass template (expected to contain 2 mg Glibenclamide) and attached to a glass slide using silicone adhesive. This adhesive was superior to other adhesives due to its capacity to maintain adhesion of the film to the glass support; its water repellency provides secondary assurance of only single surface release in addition to its noninteracting compatibility with the film. This slide was placed in the bottom of a 250 ml beaker containing 200 ml of phosphate buffer adjusted at a pH of 7.4. The beaker was kept in circulating water bath (Thermostatic shaker water bath, Julabo, Germany) adjusted at a speed of 100 rpm and the temperature was maintained at 37ºC (22). Samples were withdrawn periodically from the beaker at 15, 30, 60,120,180, 240,300 and 360 min and analyzed for drug content at 230 nm for Glibenclamide using phosphate buffer of pH 7.4 as blank. The concentration of the released drug was obtained from a previously constructed calibration curve.

In vivo studies

Healthy wistar albino male rats, weighing about 150-200g were used for the study (rats were obtained from Faculty of Veterinary Medicine, Zagazig University, animal breeding center, Egypt and treated according to Ethical committee of animal handling in Zagazig University “ECAHZU”). They were kept in clean cages and maintained in a well ventilated room with 12 hour light, 12 hour dark cycle with 25 ± 1 ºC and 45- 55% ± 5% humidity. The blood glucose Level was determined for all the rats and the fasting blood glucose level for rats was (50-80 mg/dl). Rats with lower or higher blood glucose deviating from that limit were rejected as they considered normally hypoglycemic or hyperglycemic to overcome interference of results. The animals were fed with standard laboratory diet and water. For experimental purpose, about 9 cm^2 of skin on the dorsal side of the rat was shaved by an electrical hair clipper and then washed with distilled water on the previous day of the experiment. The animals were then kept fasting overnight but allowed for water access.

The rats were divided into the following groups of 6 animals each:

Group I: Controls (didn't receive any Alloxan for induction of diabetes or any treatment)

Group II: Diabetic (induced with diabetes and didn't receive any treatments)

Group III: Diabetic rats which were transdermally treated with a matrix film (HPMC: Ed (1:1), PEG 200 and Methylene chloride: Methanol (1:1)) loaded with Glibenclamide dose (5 mg/kg) and treated transdermally.

Group IV: Diabetic rats treated with Glibenclamide suspension prepared in distilled water (5 mg/kg).

Induction of diabetes

Diabetes was induced by injection of a freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg body weight) via intraperitoneal route[23]. The rats were rested for 12 days throughout they had free access to food and water in order to maintain and ensure that they become diabetic.

Application of formulations

The formulations were applied to classified groups of animals orally using a round tipped stainless steel needle attached to 1 ml syringe and transdermally in which the film applied surface is cut to 2×2 cm² which contain a concentration of Glibenclamide equal to 5 mg/kg then it fixed on the shaved area of rat skin with the help of an adhesive tap. The dose of 5 mg/kg was selected by conducting a series of experiments with graded doses ranging between 1 to 10 mg/kg. The blood glucose level (BGL) was monitored after alloxanization in which blood samples were collected from the tail vein at different time intervals (0, 1, 2, 4, 6, 8, 10 ,12 and 24 hr) and BGL was measured using a Glucometer (Bionime, GM100, Japan). Rats with fasting glucose ranging from 180-220 mg/dl and clear signs of polyuria, polyphagia and polydipsia were considered diabetic. Animals with fasting blood glucose less than 180 mg/dl were rejected. The results were represented by plotting the % increase in BGL against time. The % increase in BGL was calculated according to the following equation:

$$\%\ \text{increase in BGL} = (\text{BGL}_{\text{measured}} - \text{BGL}_{\text{of diabetic control}} / \text{BGL}_{\text{of diabetic control}}) \times 100$$

Statistical analysis:

Results were represented as the mean±SD (n=3 for in vitro experiments and n=6 for in vivo experiments) and statistical analysis of the data were carried out using one way ANOVA followed by LSD test at a level of significant of $P < 0.05$.

RESLUTS AND DISSCUSSION

The first and most important parameter for the development of a polymeric film is the choice of the polymer. Besides having good film-forming properties and being a non-skin-irritant, the polymer must be soluble in the preparation main solvent[24]. The investigated polymers comprised two hydrophilic synthetic polymers (HPMC) and (Eudragit S100) used as individual polymers or in combinations for film formation. Apart from polymers, plasticizers such as PEG 200, PEG 400 or triacetin exerted a strong influence on the properties of the formed films[25]; resulting in higher flexibility, reduced brittleness, increased strength, improved adhesiveness of the film with other surfaces or membranes, improved the drug dissolution and changed permeability of the films[26,27].

For each polymeric film, different plasticizers were examined to obtain the best suitable and aesthetically acceptable film properties for topical application allowing attaining targeted therapeutic effect and patient compliance. The prepared Glibenclamide films were characterized based upon their physicomechanical characteristics (Table 2-4) such as thickness, folding endurance, tensile strength, elongation percent, percentage moisture loss and uptake , moisture absorption capacity ,WVT rate, drug content and weight variation. Before pouring the final prepared film components, different parameters were examined such as polymer solution viscosity, drying time, outward stickiness, cosmetical attractiveness and integrity, therefore they were ranked. The prepared films were thin, flexible, smooth and transparent in appearance, uniform in thickness and mass and showed no visible cracks.

The choice of suitable volatile solvent for Ed S100 and HPMC films was dependant on the solubility of the polymer in the chosen solvents [24]. Acetone and methanol were used for Ed S100 films while Methylene chloride was used for HPMC films and a mixture of methanol/methylene chloride was used for HPMC/ Ed S100 mixture.

Table 2. The physic-mechanical properties of the prepared films.

Formulation code	Thickness in mm ± S.D	Folding Endurance ± S.D	Tensile Strength (kg/cm²) ± S.D	Elongation % ± S.D
F1	0.17 ± 0.004	167± 13.1	0.432 ± 0.016	50 ± 0.059
F2	0.19 ± 0.001	191± 17.3	0.365 ± 0.013	38 ± 0.061
F3	0.16 ± 0.009	184± 14.7	0.471 ± 0.014	48 ± 0.049
F4	0.18 ± 0.011	197± 11.4	0.344 ± 0.008	40 ± 0.09
F5	0.1 ± 0.005	177± 12.5	0.513 ± 0.017	30 ± 0.068
F6	0.13 ± 0.0013	153± 16.2	0.382 ± 0.005	36 ± 0.063
F7	0.11 ± 0.008	64± 4.7	0.112 ± 0.003	18 ± 0.052
F9	0.08 ± 0.006	61± 3.2	0.147 ± 0.024	20 ± 0.033
F10	0.15 ± 0.011	152± 14.5	0.499 ± 0.011	26 ± 0.058
F11	0.18 ± 0.013	149± 15.4	0.478 ± 0.006	32 ± 0.091
F12	0.14 ± 0.004	138± 13.3	0.486 ± 0.017	44 ± 0.088
F13	0.12 ± 0.007	127± 12.9	0.229 ± 0.02	50 ± 0.048
F14	0.13 ± 0.009	133± 11.4	0.301 ± 0.012	28 ± 0.083
F15	0.19 ± 0.013	129± 10.7	0.251 ± 0.016	34 ± 0.077
F16	0.1± 0.0011	178± 12.2	0.401 ± 0.009	52 ± 0.082
F17	0.18 ± 0.008	184± 16.1	0.464 ± 0.018	40 ± 0.065
F18	0.11 ± 0.012	198± 18.3	0.433 ± 0.023	54 ± 0.058

The thickness of films were in the range of 0.08 mm to 0.19 mm which was found to be least for films prepared with HPMC polymers but higher for other films containing Ed S100. The folding endurance values were found to be optimum and therefore the films exhibited good physical and mechanical properties. It was found to be high for F18 (198±18) and low for F9 (61±3.2). The folding endurance was of increased value when using Ed S100 and smaller values as HPMC incorporated. The results of tensile strength showed that films made of HPMC only or having HPMC in high amounts were of less tensile strength. Also, there was an increase in the tensile strength with increasing Ed S100 concentration. Therefore, the measurements of F5, F10 and F12 gave the maximum values (0.513 ± 0.017, 0.499 ± 0.011, and 0.486 ± 0.017 kg/cm^2, respectively). On the other hand, the measurements of F7, F9 and F13 showed the least values (0.112 ± 0.003, 0.147 ± 0.024 and 0.229 ± 0.02 kg/cm^2, respectively). The percent of elongation of the prepared films was all in optimum ranges (from 18% ± 0.052 to 54% ± 0.058) (Table 2).

The films were also evaluated for the percentage moisture uptake (PMU) and percentage moisture loss (PML).The observed PMU was in the following order: F7>F9>F13>F14>F15>F17>F18>F16>F11>F10>F12>F2>F6>F4>F5>F1>F3. The previous order revealed that PMU was increased with increasing the concentration of hydrophilic polymer (HPMC). The moisture uptake of the transdermal formulations was in ranges of (2.65% to 25.94%) which considered low enough to protect the formulations from microbial contamination and reduced bulkiness of films. On the other hand, PML results were ordered as follows: F9>F7>F13>F14>F15>F16>F17>F18>F12>F4> F3>F11>F1>F6>F5>F10> F2. The previous result could be due to the high degree of hydration of the polymer HPMC, hence, the film having HPMC as 100% showed higher PML than formulations containing the hydrophobic polymer Ed S100 [28]. The moisture loss of the transdermal formulations was in ranges of (3.60% to 9.56%).

Polymer swelling in the water absorption capacity tests permits a mechanical entanglement by exposing the sites for hydrogen bonding and/or electrostatic interactions[29]. However, a critical degree of hydration of the adhesive polymer exists where optimum water absorption occurs[30]. The addition of the water-insoluble drug (Glibenclamide) increased the water uptake of the film. This is possibly as a result of the micronized drug particles existing between the polymer chains and allowing each chain to hydrate freely where weak hydrogen bonding areas were created around the Glibenclamide molecules[31]. These areas may increase the strength of the swollen layer followed by an obvious increase in the amount of penetrated water. The comparative water absorption percentage for various formulations was in the order: F14> F15> F13 > F17> F2 > F6> F11> F16> F1> F18> F4> F5> F12 > F10> F3.

The percentage swelling of HPMC films was reduced by the addition of Ed S100. HPMC containing films showed higher percent swelling due to the presence of more hydroxyl group in the HPMC molecules and the films containing HPMC as 100% were solubilized in water after only an hour. The water absorption capacity of the prepared transdermal films were low (41.37% to 133.21%), which could help the formulations remain stable, not completely dried and of reduce brittleness during storage. This water capacity is believed to affect drug release from controlled-release matrices[32]. The consequence of water uptake could be the formation of empty spaces within the film matrix that could make its structure less resistant to mechanical stresses [27].

WVT studies indicated that all the films were permeable to water vapor. WVT were high in case of F4 (108.22 mg/cm/72 hrs) and low in case of F9 (24 mg/cm/72 hrs). WVT was affected by film thickness as it was increased with increasing the film thickness and vice versa[33].The WVT was found in the order: F4 >F2> F6> F1>F3> F5> F15>F11> F17> F10> F12>F14>F18>F13 >F7> F16>F9 (Table 3).

Table 3. The physicomechanical properties of the prepared films.

Formulation code	Percentage Moisture Loss ± S.D	Percentage moisture uptake % ± S.D	Water absorption capacity % ± S.D	WVTR mg/cm/72 hrs ± S.D
F1	4.35 ± 0.006	3.39 ± 0.003	62.28 ± 0.072	80.48 ± 0.092
F2	3.60 ± 0.004	6.22± 0.017	80.27 ± 0.056	90.36 ± 0.073
F3	5.25 ± 0.012	2.65 ± 0.001	41.37 ± 0.028	79.06 ± 0.085
F4	5.52 ± 0.009	5.38 ± 0.007	60.83 ± 0.039	108.22 ± 0.066
F5	3.79 ± 0.003	4.19 ± 0.008	47.62 ± 0.042	63.68 ± 0.043
F6	4.21 ± 0.011	6.11 ± 0.014	73.41 ± 0.054	80.64 ± 0.075
F7	8.56 ± 0.003	25.94± 0.001	Solubilized	28.6 ± 0.037
F9	9.56 ± 0.012	24.93± 0.011	Solubilized	24 ± 0.015
F10	3.72 ± 0.009	7.57 ± 0.006	46.41 ± 0.015	39 ± 0.023
F11	4.39 ± 0.007	9.14 ± 0.003	72.84 ± 0.033	46.8 ± 0.049
F12	5.98 ± 0.011	7.61± 0.012	46.67 ± 0.014	35 ± 0.074
F13	8.04 ± 0.009	24.89± 0.002	108.65 ± 0.061	28.8 ± 0.057
F14	7.74 ± 0.012	21.21± 0.005	133.21 ± 0.079	33.8 ± 0.064
F15	7.46 ± 0.010	19.59± 0.008	102.67 ± 0.065	51.3 ± 0.095
F16	7.36 ± 0.008	10.02± 0.013	67.86 ± 0.032	28 ± 0.033
F17	6.83 ± 0.006	12.99± 0.007	90.08 ± 0.058	41.4 ± 0.071
F18	6.63 ± 0.011	12.93± 0.012	62.20 ± 0.018	33 ± 0.046

The drug content determination revealed an even distribution of Glibenclamide throughout the films, and the drug content was found to be in the ranges between 96.49 % and 99.81%. The weight variations were found to be in the range 22.78 mg to 39.41 mg (Table 4).

Table 4. The drug content and weight variations of the prepared films.

Formulation code	Drug content (% ± S.D)	Weight variation (mg ± S.D)
F1	97.45 ± 0.108	27.26 ± 0.159
F2	98.39 ± 0.113	31.84 ± 0.321
F3	99.48 ± 0.212	24.87 ± 0.283
F4	97.38 ± 0.163	27.53 ± 0.296
F5	98.96 ± 0.204	25.29 ± 0.264
F6	97.84 ± 0.146	31.81 ± 0.192
F7	96.49 ± 0.135	23.44 ± 0.098
F9	97.88 ± 0.139	22.86 ± 0.147
F10	97.36 ± 0.157	39.41 ± 0.125
F11	98.42 ± 0.131	36.63 ± 0.237
F12	98.31 ± 0.087	28.64 ± 0.218
F13	99.28 ± 0.142	25.71 ± 0.305
F14	98.29 ± 0.095	28.99 ± 0.288
F15	98.22 ± 0.084	26.55 ± 0.247
F16	99.37 ± 0.118	22.78 ± 0.189
F17	99.42 ± 0.105	24.53 ± 0.233
F18	99.81 ± 0.098	23.73 ± 0.317

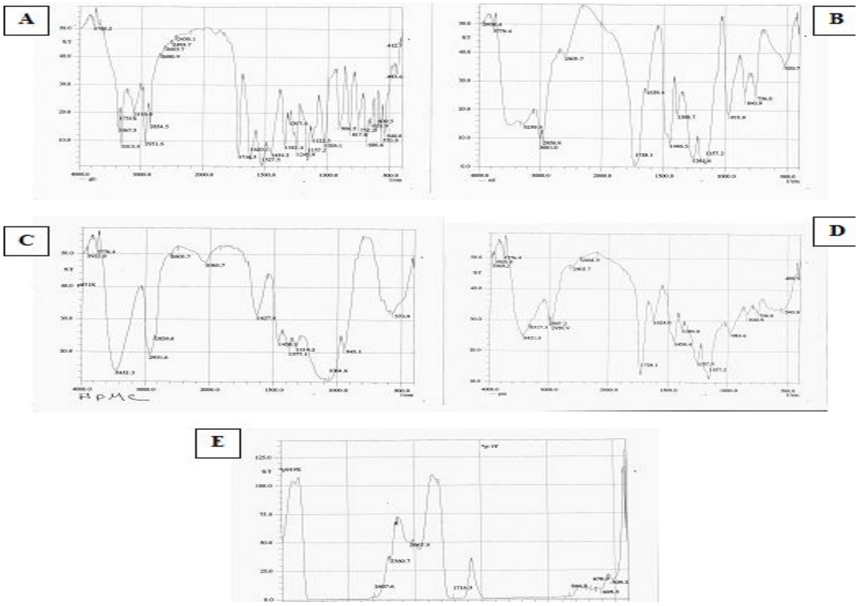

Figure 1. FTIR spectra of (A) pure Glibenclamide, (B)pure Eudragit S 100, (C) pure HPMC 2910 E5 , (D) Physical mixture of Glibenclamide : Eudragit S 100 : HPMC (0.25: 1:1) and (E) F 18.

The FTIR study was conducted to examine if an interaction between Glibenclamide and the polymers in the solid state could occur. The FTIR spectrum of pure Glibenclamide (Figure 1) showed characteristic amide peaks at 3367.5, 3313.5 and 1716.5 cm^{-1}, urea carbonyl stretching (urea N-H stretching) vibrations at 1620.1 and 1527.5 cm^{-1}, SO_2 stretching vibrations at 1157.2 and 1342.4 cm^{-1}. Figure 1 illustrates the IR spectra of pure Glibenclamide, Eudragit S 100, HPMC, Physical mixture of them and F 18. The characteristic bands of the drug were also apparent in Physical mixture such as amide peak at 1728.1 cm^{-1}, urea N-H stretching vibrations at 1624 cm^{-1} and SO_2 stretching vibrations at 1157.2 cm^{-1} and with no new bands are observed in the spectrum, this will give indication about no physical interaction was occurred between drug and polymers. In F18 spectrum, a drug characteristic peak was observed at 1716.5 cm^{-1} (amide peak), which confirms with absence of new bands that no new chemical bonds were formed between the drug and the polymers [34].

It is clear from DTA thermograms in figure 2 that the drug was in a highly crystalline structure with a sharp endothermic peak at 173.29 ºC (ΔH = -336.55 m J) corresponding to its melting point but this peak could not be found from the thermograms of Physical mixture and F18 .the peak in the physical mixture is become more smaller and broader due to presence of water in HPMC which indicates decrease intensity of crystalline nature[9] and somehow shifted due to polymers used in the mixture. the results showed that the crystalline form of Glibenclamide transformed into amorphous state in F 18 and this may be due to the process of dehydration and vaporization during film preparation which may enhance the transformation of crystalline state to amorphous state [35]. It was believed that the amorphous state have higher energy with increased surface area that results into higher solubility, dissolution rates and bioavailability due to reduction of its particle size and increase its wettability by the polymers. The thermogram of F18 shows that there is no interaction between polymer and drug.

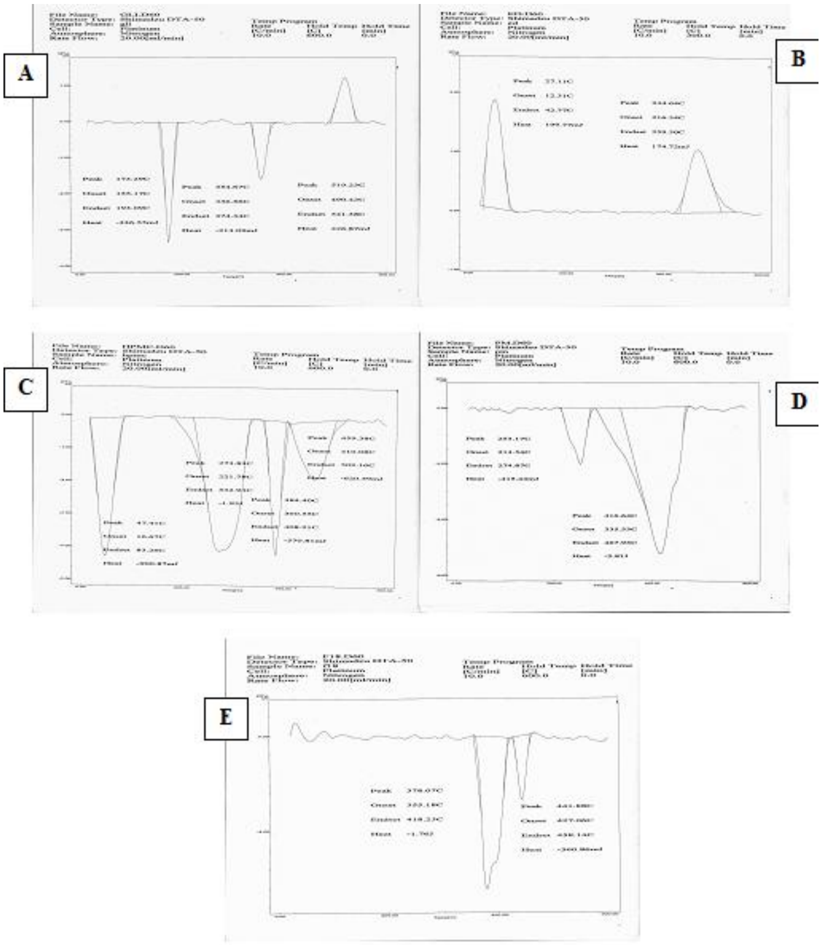

Figure 2. DTA thermogram of (A) pure Glibenclamide, (B)pure Eudragit S 100, (C) pure HPMC 2910 E5 , (D) Physical mixture of Glibenclamide: Eudragit S 100 : HPMC (0.25: 1:1) and (E) F 18.

In vitro release studies:

This study was designed to formulate a TTS able to deliver Glibenclamide in a therapeutic level using a polymeric matrix film. This allows controlling the overall release of the drug via an appropriate choice of polymers and their blends. The several diffusion pathways created due to the blend of the polymers to generate overall desired steady and sustained drug release from the films. The manner by which drug release in most of the controlled/sustained release devices including transdermal films is governed by diffusion [36, 37]. Diffusion is naturally a probabilistic process described by the random walk of molecules. The polymer matrix has a strong influence on the diffusivity of the drug as the motion of a small molecule is restricted by the three dimensional network of polymer chains. When this matrix film comes into contact with an *in-vitro* fluid, the fluid is absorbed into the polymer matrix and this initiates polymer chain dissolution process in the matrix. Polymer chain dissolution from the matrix surface involves two distinguishable steps [38]. The first step involves changes in entanglement of individual drug molecules at the matrix surface, which depends on the rate of hydration. The second step involves the shift of this molecule from the surface across the diffusion membrane initially to the surface and then to the bulk of the in vitro study fluid. *In-vitro* release profile is an important tool that predicts in advance how the drug will behave *in-vivo* [39].

A distinguishable difference was observed in the release of Glibenclamide from films containing HPMC and Ed S100 (Figure. 3). The observed results were indicating that incorporation of HPMC in the prepared films resulted in increased release characteristics of Glibenclamide due to hydration and excessive swelling percentage of the HPMC polymer (F10, F16, and F18 released about 100% after 6 hours). This may be due to dissolution of the aqueous soluble fraction of the film, which leads to creation of pores and decrease of mean diffusion path length of the drug molecule to be released [40]. The release of Glibenclamide was also high from Ed S100 films formed using methanol solvent (F2 and F6 released about 100% after 6 hours). The result could be ascribed to the higher evaporation rate of methanol solvent compared to acetone which may create invisible channels that increase drug wettability and dissolution rate.

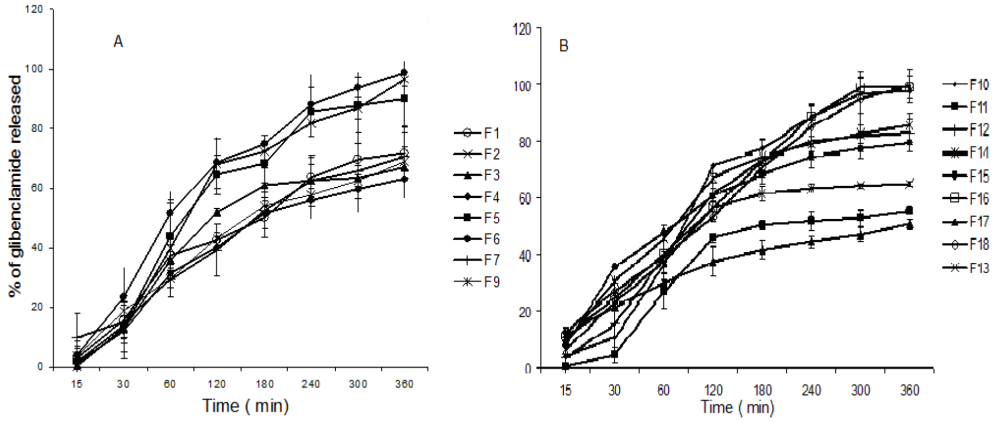

Figure 3. The cumulative release of Glibenclamide from different films.

Moreover, PEG 200 and PEG 400 were better as plasticizers in comparison to triacetin in relation to the properties of Ed S100 and HPMC types. It had been shown that the addition of PEG plasticizers led to less dense polymeric networks because of an increased mobility of the polymeric chains and the free volume between the chains. This can cause the polymeric network to relax. Therefore, the consequences of the plasticizing action of PEG are favorable to the properties of the films [41]. Finally, the drug release rate was clearly affected by the polymer type, solvent used and plasticizer. It is well known that PEGs are more hydrophilic than triacetin which has a water solubility of only 70 g/L[42]. As a result, the drug release was greater from films containing PEGs than those containing triacetin as plasticizers.

It is evident that the F18 prepared with Ed S100: HPMC (1:1) have better tensile strength, percent elongation, folding endurance and most importantly in vitro release of about 100% of the drug in 6 hours. Consequently, F18 was used for further in vivo evaluation of hypoglycemic effects of the drug.

In vivo effects of Glibenclamide film device

Based on the in vitro results of the physicochemical parameters, f18 were chosen for evaluation of *in vivo* anti-diabetic study using standard animal models. Induction of diabetes by alloxan caused a significant increase ($P < 0.001$) in the blood glucose level of experimental animals compared with non diabetic rats (control).When Glibenclamide was administered orally in suspension form (group IV), a rapid reduction of 25.69% in BGL was observed within 2 hours. On the other hand, Glibenclamide film caused a reduction in BGL slowly which reached about 30.24 % within 8 hours as shown in (Figure. 4). This reduction in BGL was sustained over long periods of time. Kahn and Shechter have suggested that a 25% reduction in blood glucose level is considered a significant hypoglycemic effect [43]. The sustained hypoglycemic effect which observed in film preparation maybe due to slow release of the drug and absorption of Glibenclamide over longer periods of time. By reweighing the experimental animals after 24 treatments with different formulations, a significant change in body weight was observed (Table 5).

Table 5. The difference in body weights after treatment.

Groups	Initial Weight	Final Weight
I	162.97 ± 1.61	181.07 ± 3.55
II	185.72 ± 2.89	177.42 ± 2.45
III	184.07 ± 3.59	186.98 ± 1.58
IV	166.92 ± 2.76	167.17 ± 4.90

There is a variance in results between those remaining diabetic and those treated even orally or transdermally. The diabetic control group rats showed significant loss of body weight ($P<0.05$). The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins which is a significant sign of diabetic rats [44]. On the other hand, animals treated with film formulation of Glibenclamide showed significant prevention of the loss in body weight throughout the study. This prevention of loss in body weight may be due to the increased glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein.

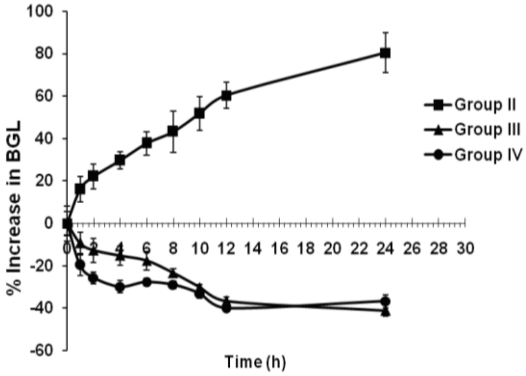

Figure 4. **Percentage increase in blood glucose levels after application of F18 transdermal film (group III) compared with oral drug administration (group IV) and untreated group (group II). The average BGL at the start of the experiment was 207.3 ± 9.7 mg/dl. Group I was normal rats with no treatments.**

CONCLUSION

Glibenclamide holds good promise for administration via transdermal route for the treatment of diabetes with an added advantage of circumventing the hepatic first pass metabolism. The various physicochemical parameters that were evaluated help to understand the usefulness and suitability of Glibenclamide to be formulated as a transdermal film with different of polymers and plasticizers. The *in vivo* study demonstrated significant hypoglycemic activity of Glibenclamide film preparation. Considering the study results polymer based films containing Glibenclamide can emerge out as an efficient drug delivery system for the treatment of Diabetes in near future.

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